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THE EFFECT OF BLUE, GREEN, RED AND WHITE LIGHT OF VARYING INTENSITIES ON THE PIGMENT CONTENT AND PHOTOSYNTHETIC CAPABILITIES OF A RED ALGA, PORPHYRA UMBILICALIS

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ABSTRACT

Freshly collected Porphyra umbilicalis plants were cultured in blue, green, red and white light at six different intensities. Cultures were maintained at 10° C with a 16 hour day length. The plants were subsequently analyzed for pigment contents and ratios and for photosynthesis capabilities. Pigment concentrations were determined by spectrophotometry. Photosynthesis rates were measured using manometric techniques. The pigment concentrations varied with the intensity of light only when red or white light was used. Plants grown in blue and green light were not as sensitive to the intensity. The ratios of pigments to each other were altered by the quality of light. The growth rates of plants were highest in red and white light cultures. Photosynthesis rates were highest in plants from the blue and green light cultures and lowest in the white and red light cultures. The plants cultured in low light intensity with all four qualities, consistently had higher photosynthesis rates than the ones cultured in high intensity light. The higher rate was due presumably to higher pigment concentrations and a better balancing of the two photosystems.

INTRODUCTION

The current study was conducted to determine the effect of various light qualities and intensities on the photosynthetic pigment content and on the photosynthesis rate in Porphyra umbilicalis (L.) J. Agardh a marine red alga in the Bangiales. Previous studies (2, 3, 12, 13, 14) on unicellular and multicellular algae have been conducted using various filters and monochromatic lights to determine their effect on the ratio of photosynthetic pigments. The results have generally confirmed Engelman's theory of "complementary chromatic adaptation". However, much controversy has developed concerning light intensity or light quality as the controlling variable (21). Following the discovery of the "enhancement effect" by Emerson (5) and the acceptance of two light reactions for photosynthesis, the accessory pigments took on new significance as collectors and conveyors of light quanta. Since light for both photosystems is necessary for normal photosynthesis, and accessory pigments function primarily in photosystem II (PS II) and chlorophyll a in photosystem I (PS I), the balance of the two systems is important for achieving maximum photosynthesis.

In this study the ratios of chlorophyll a/phycobilins (CHL/PHY), phycoerythrin/phycocyanin (PE/PC), chlorophyll a/carotenoids (CHL/CAR) were determined, as well as the total concentrations of the chlorophyll and phycobilin pigments. By determining the total concentration of the pigments, the shift in ratios caused by a particular light quality and/or

intensity can be attributed to a particular pigment. In this manner the pigments most sensitive to the qualities and the intensities of light used can be discovered. In addition, photosynthesis rates and growth rates in blue, green, red and white light were also determined. These rates could then be compared with pigment concentrations and ratios and the balancing of PS I and PS II.

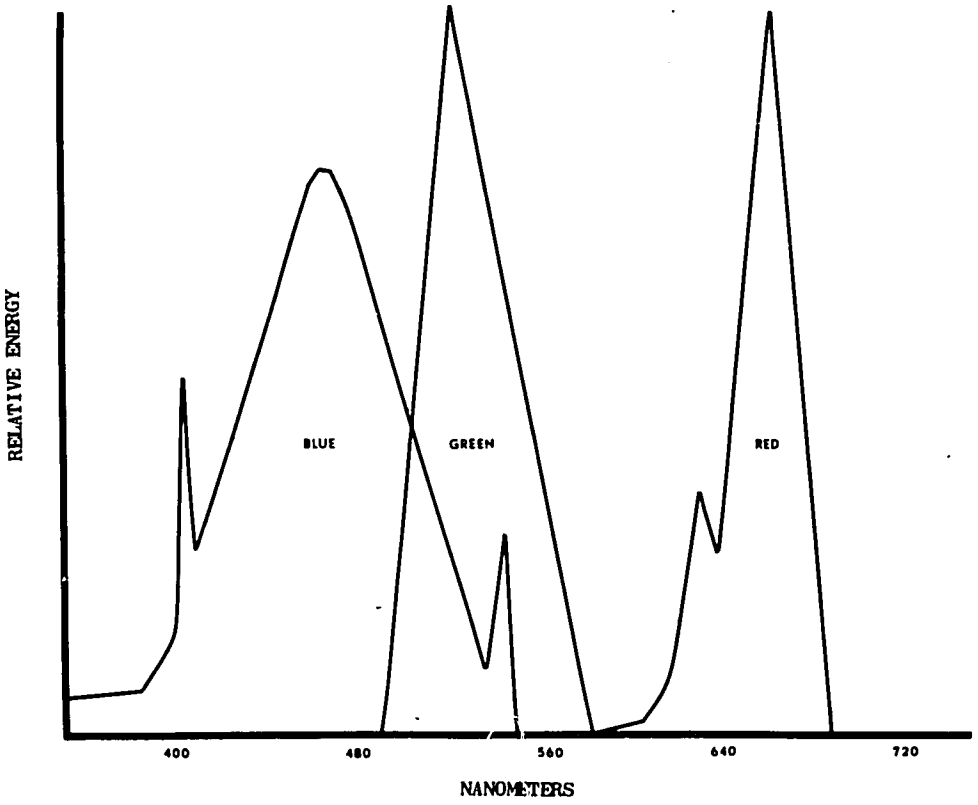
METHODS

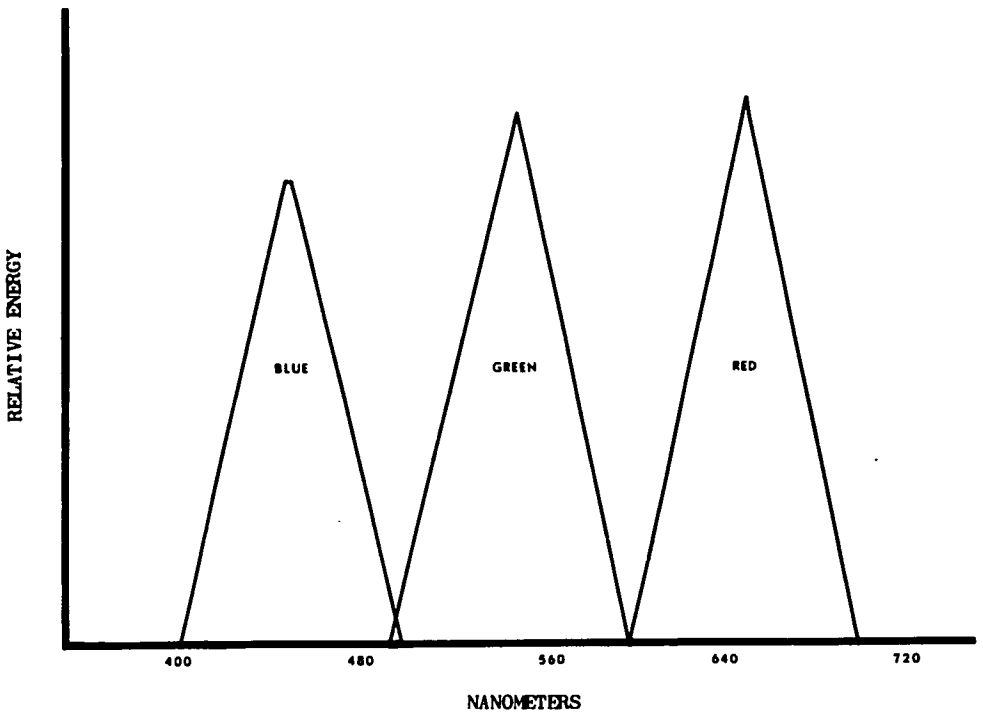
Culture set-up. In all studies Porphyra umbilicalis plants were collected from Hilton Park, Dover Point, Dover New Hampshire. Part of the fresh samples were lyophilized immediately for subsequent pigment extraction and analysis. Discs of tissue were cut from the fresh thallus using a No. 6 cork borer and these were used for determination of chromatic adaptation, photosynthesis and growth measurements. The discs were rinsed with sterilized F/2 medium (11) and placed in sterilized petri dishes in 50 mls of fresh F/2 medium. The medium was changed every week during the course of each study.

The blue, green and red regions of the spectrum were isolated using gelatin filters purchased from Kleigel Brothers., Company of Long Island City, New York. The spectral distribution of the filters is shown in Figure 1. Figure 2 shows the spectral distribution of The Carolina Biological Supply Co. (CBS) gelatin filters (20). The CBS filters were used only for photosynthesis measurements in monochromatic light as the Kleigel Brothers filters were better suited for culture use. The light energy from a mixture of Sylvania Cool White fluorescent (4) and incandescent lights was measured using an Eppley thermopile and Kintel micro-volt galvanometer. Energies were adjusted by layers of screening in conjunction with the filters. All cultures were kept in a walk-in growth chamber maintained at 10° C. The day length was 16 hours. Six different light intensities were used, as three light regimes:

Figure 1. Energy transmission of Kleigel Bros. blue, green and red gelatin filters.

Figure 2. Energy transmission of CBS blue, green and red gelatin filters.


RELATIVE ENERGY
BLUE
GREEN
RED
400
480
560
640
720
NANOMETERS


RELATIVE ENERGY
BLUE
GREEN
RED
400
480
560
640
720
NANOMETERS

Regime 1. Low = 400 $\mu w/cm^2$; high = 800 $\mu w/cm^2$

Regime 2. Low = 600 $\mu w/cm^2$; high = 2400 $\mu w/cm^2$

Regime 3. Low = 60 $\mu w/cm^2$; high = 1300 $\mu w/cm^2$

Discs were taken from the cultures at various intervals for pigment analysis, growth measurements and photosynthesis determinations.

Pigment extractions and estimations. Freshly collected and cultured plant material was lyophilized with liquid nitrogen before extraction. The lyophilized material was used for dry weight measurements. All extractions were in duplicate or triplicate. When necessary, plant material was stored in a desiccator in the freezer prior to extraction. For the chlorophyll extraction, ice cold, 80%, spectroanalyzed acetone was used. The plant material was homogenized with a hand homogenizer with the pestle connected to a mechanical stirrer. The homogenate was brought up to volume, transferred to centrifuge tubes and centrifuged for 20 min. at 25,000 x g in a refrigerated centrifuge. The absorption spectrum of the clear supernatant was determined on a Beckman DB-G grating spectrophotometer. Pigment estimation was accomplished by using an extinction coefficient of 100.9 from Smith and Benitz (24). All the operations were carried out in reduced light and cold.

Phycoerythrin and phycocyanin were extracted in a cold aqueous extract with 0.01 M phosphate buffer, pH 6.5. Plant material was homogenized and then centrifuged at 25,000 g for 15 minutes. The supernatant was decanted off and saved. The pellet was rehomogenized, combined with the supernatant

and brought up to volume. It was then centrifuged for 20 minutes at 25,000 x g. The absorption spectrum of the clear supernatant was determined on the Beckman spectrophotometer. Pigment estimations were made using the extinction coefficients of O'Carra (18) after correcting for absorption by particulate chlorophyll and allophycocyanin (1).

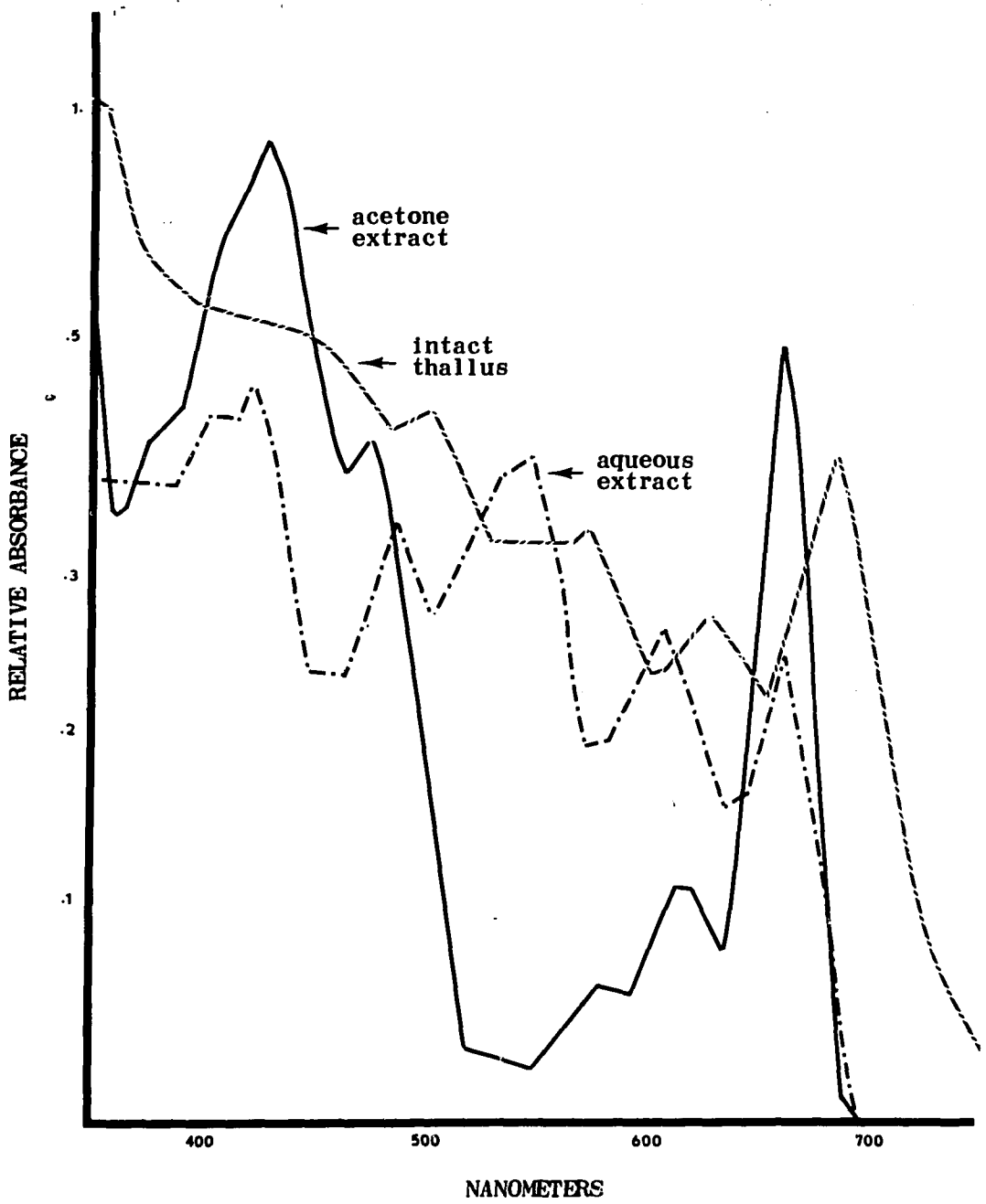
In vivo absorption spectra were determined using a tissue disc held against the inside of the cuvette by a cover slip (23). The spectra were used to compare pigment ratios determined in this manner with those obtained by the extraction procedures. The ratio of pigment classes from in vivo absorption spectra were determined as follows:

1. Ratio of chlorophyll a/carotenoid = O.D. 680/O.D. 475
2. Ratio of chlorophyll a/phycoerythrin = O.D. 680/O.D. 560
3. Ratio of chlorophyll a/phycocyanin = O.D. 680/O.D. 615
4. Ratio of phycoerythrin/phycocyanin = O.D. 560/O.D. 615

The ratios of extracted pigments were obtained by using the percentage values of each pigment. Typical absorption spectra of extracts and intact thallus are illustrated in Figure 3.

Photosynthesis measurements. Freshly cut tissue discs and cultured discs were kept overnight in the dark in Emerson's and Green's (6) artificial sea water solution. The following day the apparent photosynthesis rate in blue, green, red and white light of 500 uw/cm^2 was measured. The measurements were made using Gilson differential respirometers and

Figure 3. Absorption curves for total extracted and *in vivo* pigments.


acetone extract
intact thallus
aqueous extract
RELATIVE ABSORBANCE
1.
.5
.3
.2
.1
400
500
600
700
NANOMETERS

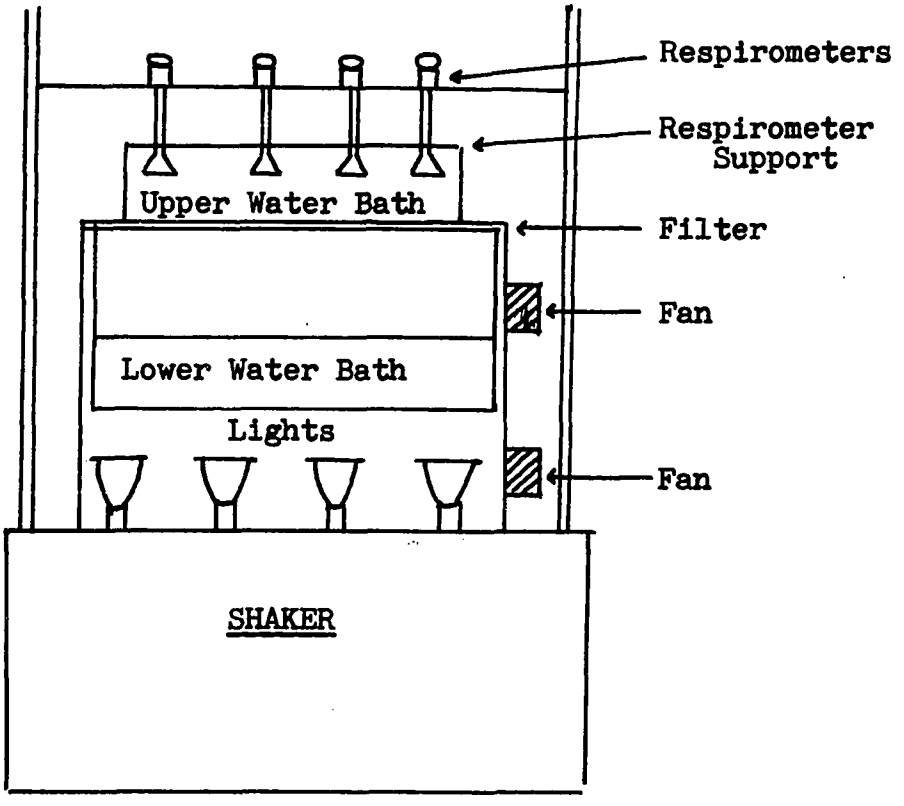


Figure 4. Diagram of experimental set-up for measuring photosynthesis rates in blue, green, red and white light.

conventional manometric techniques (27). The blue, green and red light was isolated by use of the CBS filters. The experimental setup for photosynthesis measurements is diagrammed in Figure 4. It consisted of six 50 watt incandescent flood lamps on the floor of the box. The lights were covered by a bath of 1% copper sulfate solution 5 cm. deep to absorb the far-red radiation. The solution bath was covered by the appropriate filter or clear glass for white light. On top of the filter or glass was an upper water bath in which the respirometers were swirled. The rate of swirling was 90-100 revolutions/minute. The entire apparatus was located in a constant temperature walk-in chamber. The temperature of the upper water bath was monitored during all measurements and did not fluctuate more than 0.5 degrees. The reaction flasks were covered on the sides and top with aluminum foil so that no extraneous light could enter. Two discs were used in each reaction flask. The artificial sea water was buffered with 0.015 M $KHCO_3$ and 0.001 M Na_2CO_3. Respiration rates were measured in the dark using the same apparatus except that 0.3 ml of a 20% KOH solution was added to the centerwell, and 0.4 ml to the side arm, to absorb the CO_2.

RESULTS

Pigment concentrations. The results of studies from the first intensity regime of 400 $\mu w/cm^2$ for the low intensity and 800 $\mu w/cm^2$ for the high intensity light are shown in Figure 5 and Tables 1 and 2. In the first study the pigment values were determined after 8 and 18 days in

Figure 5. Total pigment concentration of discs after 8 and 18 days in culture under blue, green, red and white light of 400 and 800 $\mu w/cm^2$.

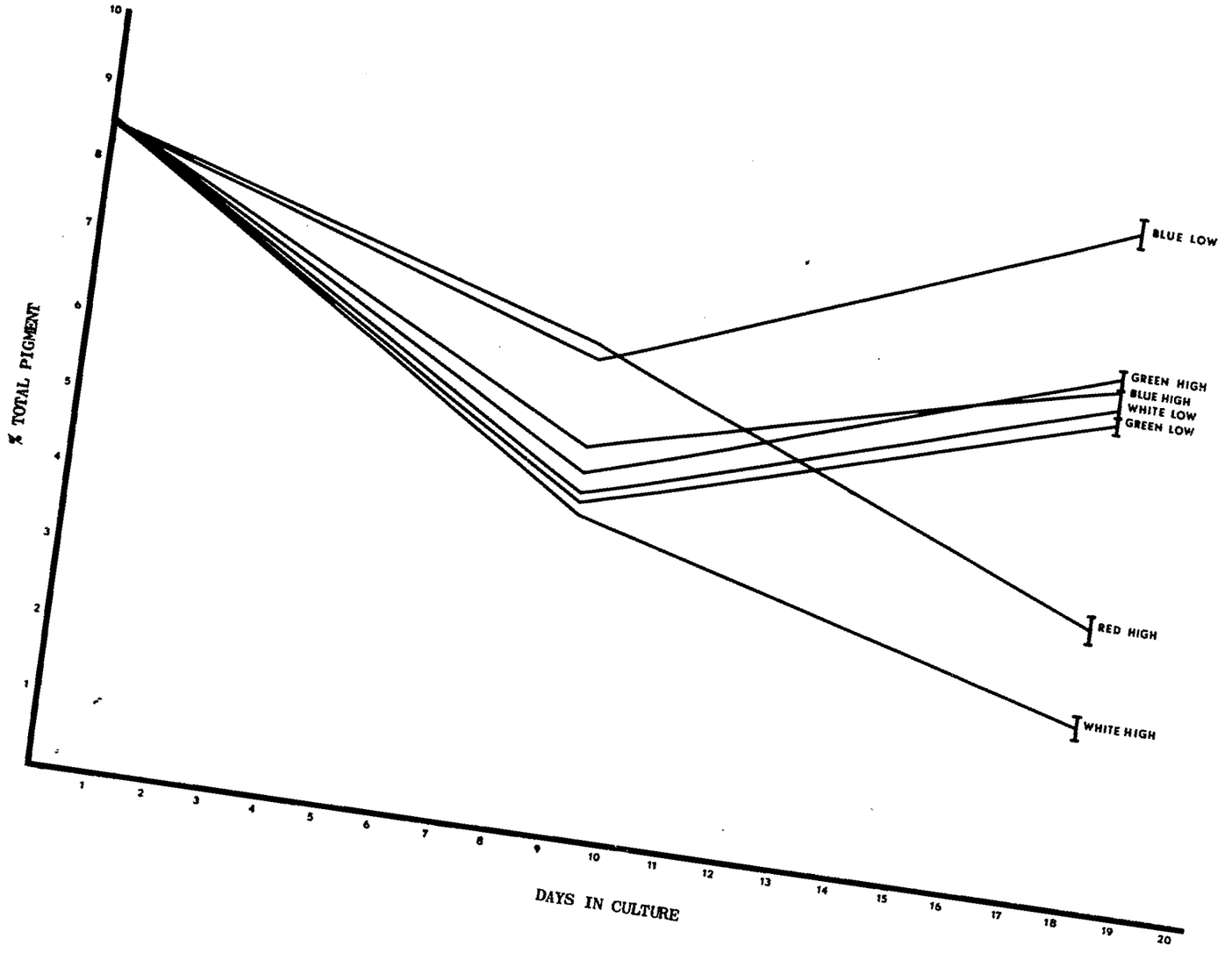

% TOTAL PIGMENT
DAYS IN CULTURE
BLUE LOW
GREEN HIGH
BLUE HIGH
WHITE LOW
GREEN LOW
RED HIGH
WHITE HIGH

Table 1. Pigment concentrations and ratios of pigment classes to each other following 8 and 18 days in culture under blue, green, red and white light. Light intensity 400 and 800 $\mu w/cm^2$.

A. 7 Days.

	RATIO			PERCENTAGE OF DISC			
CULTURE	PE/PC	CHL/PHY	CHL/CAR	CHL	PHY	TOT.	S.D.*
White high	2.44	3.58	0.534	3.33	0.93	4.26	±.14
White low	2.55	2.33	0.528	3.15	1.35	4.50	±.14
Red high	2.02	3.27	0.590	5.03	1.54	6.57	±.00
Green high	1.96	2.04	0.543	3.14	1.54	4.68	±.10
Green low	2.02	2.00	0.528	3.16	1.58	4.40	±.10
Blue high	1.74	1.84	0.545	3.57	1.94	5.51	±.00
Blue low	2.29	1.94	0.534	4.15	2.14	6.29	±.10

B. 18 Days.

	RATIO			PERCENTAGE OF DISC			
CULTURE	PE/PC	CHL/PHY	CHL/CAR	CHL	PHY	TOT.	S.D.*
White high	1.38	2,83	0.633	1.76	0.62	2.38	±.14
White low	2.04	2.22	0.543	4.59	2.07	6.66	±.00
Red high	1.70	2.15	0.675	2.49	1.16	3.65	±.10
Green high	2.08	1.92	0.548	4.60	2.40	7.00	±.10
Green low	2.87	1.70	0.533	4.10	2.35	6.45	±.00
Blue high	1.76	1.92	0.731	4.51	2.35	6.86	±.14
Blue low	2.43	2.60	0.575	6.44	2.47	8.91	±.20

* = Standard deviation. PE = phycoerythrin. PC = phycocyanin. CHL = chlorophyll. PHY = phycobilins. CAR = carotenoid. TOT. = chlorophyll and phycobilins.

Table 2. Pigment concentrations and ratios of pigment classes to each other following 30 days in culture under blue, green, red and white light. Light intensity 400 and 800 $\mu w/cm^2$.

	RATIO			PERCENTAGE OF DISC			
CULTURE	PE/PC	CHL/PHY	CHL/CAR	CHL	PHY	TOT.	S.D.*
White high	1.84	1.92	0.488	2.23	1.16	3.40	±.00
White low	2.04	2.38	0.564	4.44	1.92	6.36	±.46
Red high	1.76	2.40	0.557	4.84	2.02	6.85	±.14
Green high	2.03	2.43	0.533	5.01	2.06	7.07	±.14
Green low	2.45	1.80	0.534	4.10	2.28	6.38	±.10
Blue high	1.99	1.35	0.569	4.17	3.09	7.26	±.00
Blue low	2.19	1.55	0.520	3.66	2.36	6.02	±.00

* = Standard deviation

culture (Table 1). In a second study at these intensities the pigment values were determined after 30 days in culture (Table 2). It is evident in the results, and in the results of later studies, that with increased time in culture, under the various light intensities and qualities used, larger pigment changes occurred. The lowest pigment concentration in all experiments was found in discs from the high intensity white light cultures (White High). The total pigment concentration in discs from all other light intensity and quality cultures was similar, with the highest concentration occurring in discs from blue and green high light cultures. The ratio of chlorophyll a/phycobilins (CHL/PHY) varies considerably from one treatment to another and from high intensity and low intensity light cultures of the same quality. Although no pattern is clearly indicated by these ratios, more information can be obtained by inspection of the quantitative values. The high ratio of CHL/PHY in white high light discs after eight days in culture is accounted for by a reduction in the amounts of phycobilin pigments with little change in the chlorophyll content. The high CHL/PHY ratio of red high light discs is due to an increase in the chlorophyll content. In blue high and blue low light discs, the low CHL/PHY ratio is due to a relative increase of phycobilin pigments, with less change in the chlorophyll content. After eighteen days in culture, the white high light discs still have the highest ratio of CHL/PHY. The results show that this high CHL/PHY ratio may be attributed to a reduction of both chlorophyll and phycobilin pigments. In the discs from the blue and green

high light culture there was an increase in both of these pigments.

The ratio of phycoerythrin/phycocyanin (PE/PC) remained relatively constant. Larger differences did develop with time, however, as indicated in Tables 1 and 2. After eighteen days culturing at these intensities in one study and 30 days in another, the highest ratios were in discs from green high and low light. The lowest ratio was in discs from red high light cultures. The low light intensities, except red light, had lower PE/PC ratios than did the high light intensity discs.

The chlorophyll a/carotenoid (CHL/CAR) ratio was not altered significantly after seven days in culture. After a longer time in culture, the range of the ratios was larger with the highest ratio in discs from white, red and blue high light cultures.

The results of studies using the second intensity regime of 600 $\mu w/cm^2$ for the low intensity and 2400 $\mu w/cm^2$ for high intensity light are shown in Tables 3 and 4. The total pigment concentration after 14 days in culture was lowest in discs from the white and red high light cultures. The highest concentrations of pigments were in discs from all the low light intensity cultures and in discs from blue and green high light cultures. The CHL/PHY ratio showed very little variation at this time. The highest ratio was found in discs from the green low light culture. The quantitative values showed that there was a large reduction in chlorophyll and phycobilins in discs from white and red high light cultures.

Table 3. Pigment concentrations and ratios of pigment classes to each other following 14 days in culture under blue, green, red and white light. Light intensity was 600 and 2400 $\mu w/cm^2$.

	RATIO			PERCENTAGE OF DISC			
CULTURE	PE/PC	CHL/PHY	CHL/CAR	CHL	PHY	TOT.	S.D.*
White high	1.77	1.61	0.464	2.36	1.47	3.83	±.24
White low	1.64	1.67	0.553	4.15	2.48	6.63	±.10
Red high	1.69	1.83	0.468	2.65	1.45	4.10	±.14
Red low	1.67	1.55	0.527	3.85	2.48	6.33	±.00
Green high	1.80	1.70	0.523	3.52	2.07	5.59	±.31
Green low	1.65	1.50	0.520	3.18	2.12	5.30	±.14
Blue high	1.87	1.67	0.528	3.92	2.35	6.27	±.14
Blue low	1.72	1.66	0.533	3.57	2.15	5.27	±.00

Table 4. Pigment concentrations and ratios of pigment classes to each other following 30 days in culture under blue, green, red and white light. Light intensity was 600 and 2400 $\mu w/cm^2$.

	RATIO			PERCENTAGE OF DISC			
CULTURE	PE/PC	CHL/PHY	CHL/CAR	CHL	PHY	TOT.	S.D.*
White high	4.00	4.29	0.517	1.50	0.35	1.85	±.24
White low	3.00	2.41	0.565	2.22	0.92	3.14	±.33
Red high	2.90	3.67	0.519	1.43	0.39	1.82	±.20
Red low	2.81	2.48	0.569	2.46	0.99	3.45	±.40
Green high	2.33	3.06	0.564	2.45	0.80	3.25	±.12
Green low	3.26	2.62	0.561	2.57	0.98	3.55	±.10
Blue high	2.33	2.47	0.569	2.72	1.10	3.82	±.14
Blue low	1.98	2.16	0.567	2.90	1.34	4.24	±.14

* = Standard deviation

The ratio of PE/PC also remained quite constant. The most noticeable result was that discs from low light intensity cultures had a lower ratio than their high light intensity counterparts. The CHL/CAR ratio was consistent in all cases except in discs from white and red high light cultures which were lower than discs from the other treatments.

The study carried out for 30 days at the same intensities showed similar pigment changes but developed to a larger extent. The lowest pigment concentration was found in discs from white and red high light cultures. The pigment concentration was highest in discs from the low light intensity cultures and in discs from blue and green high light cultures. The CHL/PHY ratio showed larger variations than in the 14 day study. The highest ratio occurred in discs from white, red and green high light cultures. The discs from the low light intensity cultures had lower ratios than their counterparts from the high light intensity cultures. As in the 14 day study, the chlorophyll and phycobilin content was greatly reduced in discs from white and red high light cultures. The ratio of PE/PC also showed more variation than in the 14 day study. The highest ratio occurred in discs from white high light cultures. Except in green light, the low light intensity cultures had a lower ratio of PE/PC than their high intensity counterparts. The ratio of CHL/CAR was again different only in discs from white and red high light cultures.

The third light intensity regime used was 60 $\mu w/cm^2$ for the low and 1300 $\mu w/cm^2$ for the high light intensity cul-

tures. In the studies conducted using these two light intensities, intact thallus absorption spectra were determined in addition to the other values determined in previous studies. The spectra were used to further elucidate the variations in pigment ratios which occurred following the light quality and intensity treatments. The values were also compared to those obtained by pigment extraction. The total pigment concentrations and ratios from one study following 7 and 52 days in culture are indicated in Table 5 and Figure 6. The results show that after 7 days (Table 5A) some differences in total pigment concentrations were beginning to develop between the low and high light intensity cultures, the largest difference being evident between discs from the white high and white low light cultures. The discs from the white high light cultures had the lowest pigment concentration of any cultured discs. The blue low light discs had the highest pigment concentration. At this time, the ratio of CHL/PHY pigments was highest in discs from white low, blue and green high light cultures. The ratio of PE/PC showed that discs from blue high light cultures had the lowest ratio, and that discs from the blue low light cultures had the highest ratio. However, the range of all ratios was not large. The CHL/CAR ratio showed that discs from blue high light cultures had the highest ratio, but the ratio did not vary as much as in previous studies. Table 6 shows the results of the intact thallus absorption spectra after 7 days at these intensities. The results verify those of the pigment extraction in that the ratio of PE/PC does not fluctuate very much. The ratio

Figure 6. Total pigment concentration of discs after 7 and 52 days in culture under blue, green, red and white light of 60 and 1300 $\mu w/cm^2$.

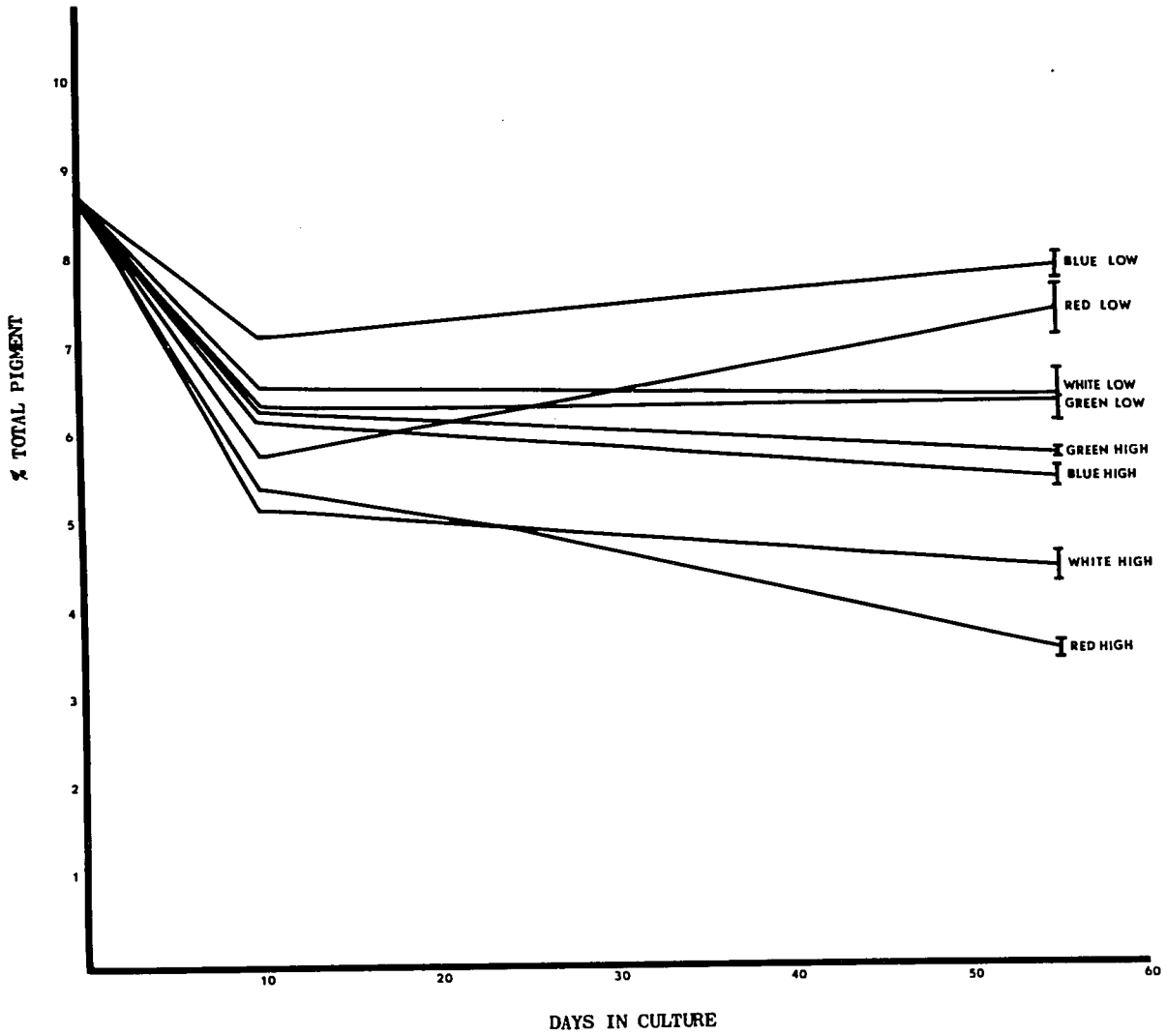

% TOTAL PIGMENT
10
9
8
7
6
5
4
3
2
1
10
20
30
40
50
60
DAYS IN CULTURE
BLUE LOW
RED LOW
WHITE LOW
GREEN LOW
GREEN HIGH
BLUE HIGH
WHITE HIGH
RED HIGH

Table 5. Pigment concentrations and ratios of pigment classes to each other following 7 and 52 days in culture under blue, green, red and white light. Light intensity 60 and 1300 $\mu w/cm^2$.

A. 7 Days.

	RATIO			PERCENTAGE OF DISC			
CULTURE	PE/PC	CHL/PHY	CHL/CAR	CHL	PHY	TOT.	S.D.*
White high	1.97	1.02	0.570	2.62	2.61	5.23	±.31
White low	1.98	1.38	0.556	3.81	2.78	6.58	±.24
Red high	2.08	1.04	0.572	2.84	2.74	5.58	±.10
Red low	2.11	1.00	0.657	2.89	2.90	5.78	±.10
Green high	2.12	1.53	0.595	3.91	2.56	6.47	±.20
Green low	2.08	1.04	0.590	3.21	3.12	6.32	±.00
Blue high	1.86	1.48	0.697	3.84	2.59	6.43	±.10
Blue low	2.22	1.01	0.575	3.60	3.56	7.16	±.20

B. 52 Days.

	RATIO			PERCENTAGE OF DISC			
CULTURE	PE/PC	CHL/PHY	CHL/CAR	CHL	PHY	TOT.	S.D.*
White high	2.09	2.77	0.523	3.32	1.21	4.52	±.14
White low	2.33	1.58	0.655	3.94	2.52	6.46	±.33
Red high	2.00	2.52	0.516	2.58	1.02	3.60	±.10
Red low	2.45	2.24	0.551	5.11	2.33	7.44	±.45
Green high	2.68	2.17	0.593	3.27	2.07	5.84	±.00
Green low	2.88	2.74	0.606	4.70	1.72	6.42	±.00
Blue high	2.64	1.73	0.578	3.55	1.55	5.60	±.14
Blue low	2.51	1.67	0.552	4.96	2.96	7.92	±.00

* = Standard deviation

of chlorophyll a absorbance/phycoerythrin absorbance had not changed much after 7 days.

After 52 days in culture (Table 5B) the total pigment concentrations had developed along the typical patterns. Discs from white and red high light cultures showed the lowest pigment content. Discs from the other six cultures were similar in pigment concentrations with discs from blue low light intensity cultures having the most. The CHL/PHY ratio varied much more than it did at 7 days. The highest ratio was found in discs from white high light cultures because of a decrease in both chlorophyll a and phycobilins. Discs from green low light cultures also had a high CHL/PHY ratio. The reason, in this case, was due to a relative increase in the chlorophyll a concentration. The lowest ratio of CHL/PHY occurred in discs from the white and blue low light cultures. The PE/PC ratio was highest in discs from green high and green low light cultures. The lowest ratio was in discs from white and red high light cultures. The CHL/CAR ratio was highest in discs from white low light cultures. Table 6 shows the results from the intact thallus absorption spectra after 52 days in culture. Again, the results verify those obtained by pigment extraction methods.

A 14 day study using 60 $\mu w/cm^2$ was conducted and the results are shown in Table 7. The discs from the blue light culture had the highest pigment concentration. The lowest CHL/PHY ratio occurred in discs from the blue light cultures. The discs from the red culture had the lowest PE/PC ratio while the highest ratio was found in discs from

Table 6. Ratio of absorbance maximum for chlorophyll a, phycoerythrin and phycocyanin to each other. The absorption maximum was obtained from intact thallus absorption spectra following culture for 7 and 52 days in blue, green, red and white light. Light intensity 60 and 1300 $\mu w/cm^2$.

A. 7 Days.

	RATIO			
CULTURE	CHL/PC	CHL/PE	PE/PC	S.D.*
White high	1.09	1.00	1.09	±.00
White low	1.08	1.03	1.05	±.02
Red high	1.10	1.02	1.09	±.01
Red low	1.08	1.01	1.07	±.00
Green high	1.13	1.02	1.10	±.00
Green low	1.08	1.01	1.07	±.01
Blue high	1.03	0.95	1.08	±.01
Blue low	1.09	1.01	1.08	±.00

B. 52 Days.

	RATIO			
CULTURE	CHL/PC	CHL/PE	PE/PC	S.D.*
White high	1.13	1.03	1.09	±.01
White low	1.06	0.94	1.13	±.00
Red high	1.18	1.11	1.06	±.01
Red low	1.08	1.01	1.07	±.00
Green high	1.05	0.95	1.09	±.01
Green low	1.07	0.97	1.10	±.01
Blue high	1.04	0.97	1.09	±.00
Blue low	1.10	1.10	1.06	±.01

* = Standard deviation

Table 7. Pigment concentrations and ratios of pigment classes to each other following 14 days in culture under blue, green, red and white light of 60 $\mu w/cm^2$.

	RATIO			PERCENTAGE OF DISC			
CULTURE	PE/PC	CHL/PHY	CHL/CAR	CHL	PHY	TOT.	S.D.*
White	1.76	1.56	0.570	3.52	2.25	5.77	±.12
Red	1.54	1.80	0.556	3.47	1.93	5.40	±.10
Green	2.05	1.70	0.548	3.85	2.26	6.11	±.14
Blue	1.98	1.45	0.600	3.59	2.47	6.06	±.20

* = Standard deviation

the green light culture. The discs from the blue light cultures had the highest CHL/CAR ratio.

Photosynthesis rates. Figure 7 shows a typical apparent photosynthesis and respiration curve for Porphyra umbilicalis in the temperature range of 1-36° C. The light intensity for the photosynthetic measurements was 4000 $\mu w/cm^2$. The photosynthesis rates in white light were measured and compared with the pigment alterations induced by the culture conditions. Figure 8 shows the photosynthesis rates in white light of 4000 $\mu w/cm^2$ determined after 8 and 18 days in culture under blue, green, red and white light of 800 $\mu w/cm^2$. The discs from the blue and green light cultures had a photosynthesis rate three to five times larger than did discs from red and white light cultures. The relative rates of photosynthesis are similar to the relative concentration of pigments in discs from each respective culture.

Figure 9 shows the photosynthesis rate of discs in white light of 5000 $\mu w/cm^2$ after culturing in light intensities of 60 and 1300 $\mu w/cm^2$. As with the other regimes, the discs from the low intensity cultures had much higher photosynthesis rates. The lowest rates occurred with discs from the red and white high light cultures.

Tables 8 and 9 show the photosynthesis rates in white light of 500 $\mu w/cm^2$. The discs had previously been irradiated with 600 and 2400 $\mu w/cm^2$ of blue, green, red and white light. The rates of photosynthesis of cultured material were determined after 18 days in one study (Table 8) and 30 days in the other (Table 9). In both studies the discs

Figure 7. Apparent photosynthesis and respiration curve of *P. umbilicalis*. Light intensity was 4000 $\mu w/cm^2$.

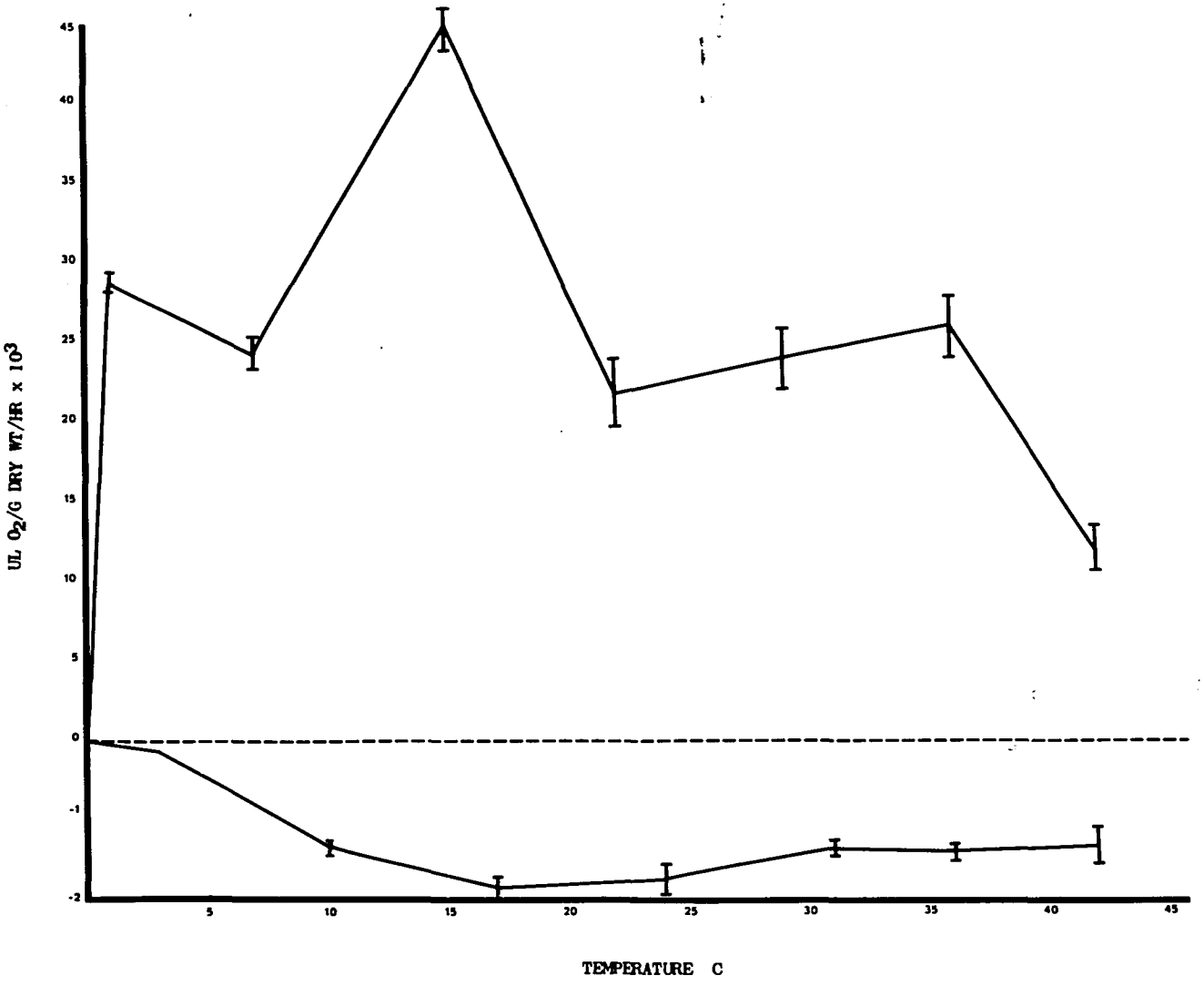

UL O_2/G DRY WT/HR x 10^3
45
40
35
30
25
20
15
10
5
0
-1
-2
5
10
15
20
25
30
35
40
45
TEMPERATURE C

Figure 8. Apparent photosynthesis rate of discs in white light of 4000 $\mu w/cm^2$ after 7 and 18 days of culture in blue, green, red and white light of 800 $\mu w/cm^2$.

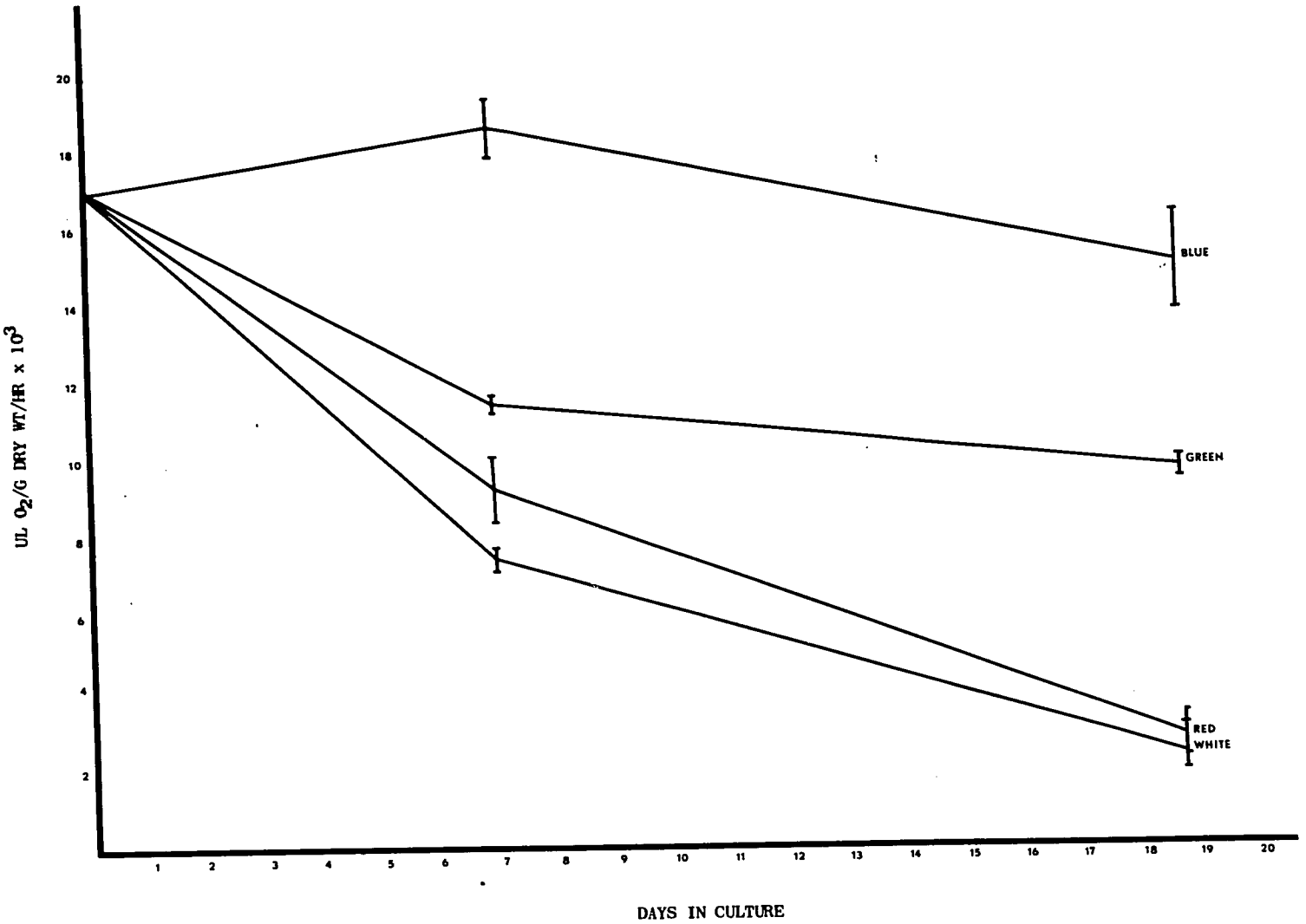

UL O_2/G DRY WT/HR x 10^3
20
18
16
14
12
10
8
6
4
2
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
DAYS IN CULTURE
BLUE
GREEN
RED
WHITE

Figure 9. Apparent photosynthesis rate in white light of 5000 $\mu w/cm^2$ after 7 and 52 days of culture in blue, green, red and white light of 60 and 1300 $\mu w/cm^2$.

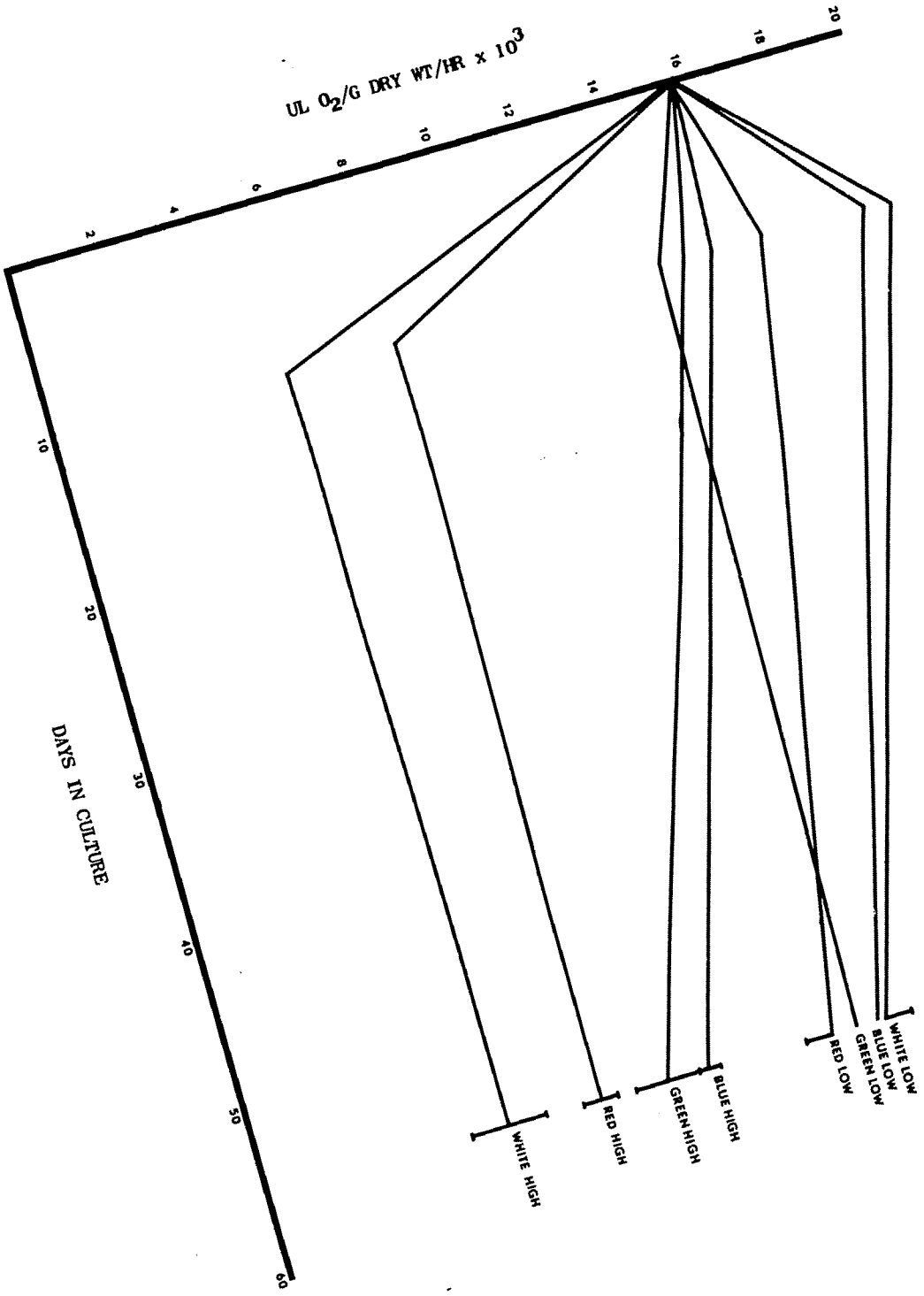

UL O_2/G DRY WT/HR x 10^3
2
4
6
8
10
12
14
16
18
20
10
20
30
40
50
60
DAYS IN CULTURE
WHITE HIGH
RED HIGH
GREEN HIGH
BLUE HIGH
RED LOW
GREEN LOW
BLUE LOW
WHITE LOW

Table 8. Mean weight(mg) of discs and their photosynthesis rates in white light of 500 $\mu w/cm^2$ following 18 days in culture under blue, green, red and white light of 600 and 2400 $\mu w/cm^2$.

CULTURE	WEIGHT(mg) OF DISC	PHOTOSYNTHESIS($\mu l\ O_2/g/hr$)
White high	30.5 ± 1.4*	1200 ± 600
White low	15.8 ± 3.2	4078 ± 429
Red high	24.3 ± 2.5	1434 ± 297
Red low	21.6 ± 1.0	7789 ± 351
Green high	20.0 ± 2.2	5122 ± 889
Green low	15.3 ± 3.4	10709 ± 1149
Blue high	14.3 ± 0.8	5340 ± 158
Blue low	10.5 ± 0.5	13325 ± 1397

Table 9. Mean weight(mg) of discs and their photosynthesis rates in white light of 500 $\mu w/cm^2$ following 30 days in culture under blue, green, red and white light of 600 and 2400 $\mu w/cm^2$.

CULTURE	WEIGHT(mg) OF DISC	PHOTOSYNTHESIS($\mu l\ O_2/g/hr$)
White high	30.0 ± 1.4*	600 ± 250
White low	28.0 ± 1.0	3173 ± 995
Red high	27.3 ± 2.1	892 ± 75
Green high	27.4 ± 0.5	1855 ± 350
Green low	22.0 ± 1.1	3406 ± 400
Blue high	24.0 ± 0.0	7324 ± 550
Blue low	22.0 ± 0.5	7704 ± 1200

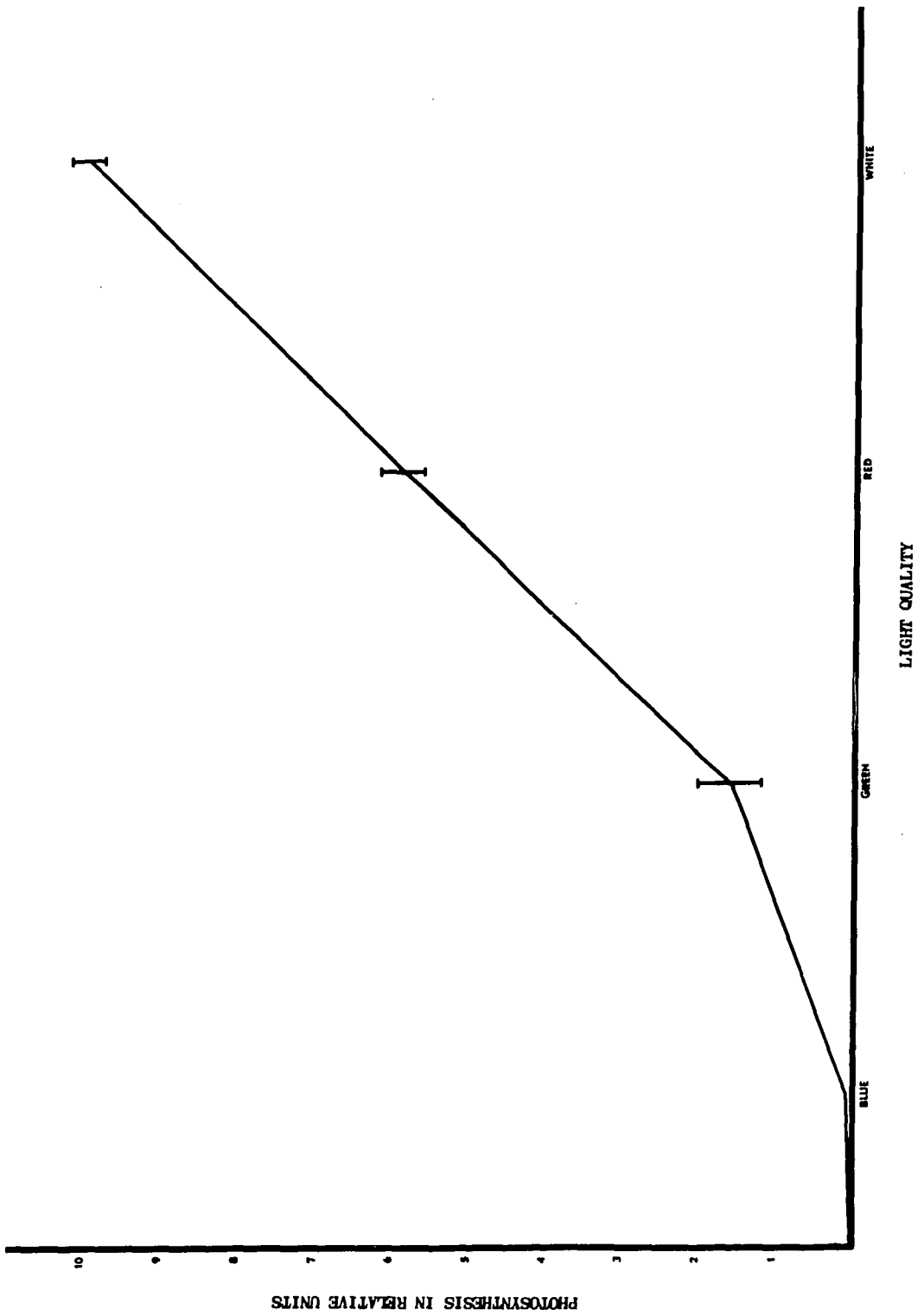
* = Standard deviation

from the low intensity cultures had much higher rates than their counterparts from the high intensity cultures. The highest rates were with discs from the blue and green low light cultures.

Photosynthesis rates in monochromatic light. The rates of photosynthesis when primarily one photosystem was being irradiated with light were measured using blue, green, red and white light as previously described. The light intensity for all measurements was 500 $\mu w/cm^2$. The measurements were made to determine if a change in the balance of the two photosystems was occurring following treatment with a particular light quality and/or light intensity. Figure 9 shows a typical curve of the rate of photosynthesis at each light quality for freshly collected plants. The rates depicted in that figure were obtained in the following manner. The apparent photosynthesis rate of 40 freshly collected samples, obtained over an 18 month period, was measured using blue, green and red light of 500 $\mu w/cm^2$. The rate in white light for each sample was divided into the rate in blue, green and red light for the same sample. The percentages obtained were then averaged and used for the graph. Using 10 as a relative unit value of photosynthesis in white light, then the rate in red light would be 6/10th as much, or 6. Similar rates were determined from discs which had been kept in culture under the previously mentioned light intensities and qualities.

The photosynthesis measurements using blue light were difficult and inconclusive because of an increase in O_2

Figure 10. Apparent photosynthesis rates of freshly collected plants in blue, green, red and white light of 500 $\mu w/cm^2$.


PHOTOSYNTHESIS IN RELATIVE UNITS
10
9
8
7
6
5
4
3
2
1
BLUE
GREEN
RED
WHITE
LIGHT QUALITY

uptake by the discs when exposed to blue light. During irradiation with blue light the O_2 uptake was usually larger than the O_2 evolution from photosynthesis. This is similar to the "respiratory enhancement" reported by several investigators (15, 25). For this reason the rates of photosynthesis in blue light will not be considered here. The rate of photosynthesis in green light, stimulating primarily PS II, and the rate of photosynthesis in red light, stimulating primarily PS I, are of major concern. These rates were divided into the photosynthesis rate in white light, when both photosystems are being stimulated, to give ratios as follows:

R^1 = photosynthesis in white light/photosynthesis in red light (white/red)

R^2 = photosynthesis in white light/photosynthesis in green light (white/green)

It should be mentioned that in measuring the photosynthesis rate using the red filter, both photosystems were being stimulated because the spectral distribution of this filter includes some of the absorption area of phycocyanin (PS II). These measurements were carried out on discs from the two intensity regimes of:

1. 600 $\mu w/cm^2$ and 2400 $\mu w/cm^2$
2. 60 $\mu w/cm^2$ nad 1300 $\mu w/cm^2$

The results of the photosynthesis measurements from the first regime are shown in Table 10. The rates in this study were measured following 14 days in culture. The ratio of white/green photosynthesis in discs from blue, red and white high light cultures were all minus values as no

Table 10. Photosynthesis rates and ratios in green, red and white light of 500 $\mu w/cm^2$ following 14 days in culture under blue, green, red and white light of 600 and 2400 $\mu w/cm^2$.

	PHOTOSYNTHESIS ($\mu l O_2/g/hr$)			PHOTOSYNTHESIS RATIOS	
CULTURE	GREEN LIGHT	RED LIGHT	WHITE LIGHT	WHITE/GREEN	WHITE/RED
White high	___ *	$528^{+}_{-}43$	$1200^{+}_{-}154$	___	2.06
White low	$1134^{+}_{-}855$	$1928^{+}_{-}102$	$4078^{+}_{-}429$	3.60	2.11
Red high	___	$789^{+}_{-}54$	$1434^{+}_{-}297$	---	1.82
Red low	$517^{+}_{-}267$	$3738^{+}_{-}1200$	$7789^{+}_{-}351$	15.01	2.08
Green high	$2344^{+}_{-}1482$	$1743^{+}_{-}477$	$5122^{+}_{-}889$	2.19	2.94
Green low	$4280^{+}_{-}962$	$3654^{+}_{-}714$	$10709^{+}_{-}1149$	2.50	2.93
Blue high	___	$1524^{+}_{-}341$	$5340^{+}_{-}158$	___	3.50
Blue low	$1300^{+}_{-}154$	$8564^{+}_{-}899$	$13325^{+}_{-}1397$	10.25	1.56

* = no apparent photosynthesis

measurable photosynthesis occurred when using the green filter. The lowest white/green photosynthesis ratio was in green low and green high light cultured discs. The highest ratio, other than the minus values, was found in discs from the red low light cultures. The ratio of white/red photosynthesis was lowest in discs from blue low and red high light cultures. Table 11 shows the photosynthesis rates and ratios of discs after 7 and 21 days of culture at 60 $\mu w/cm^2$. The most noticeable result was that all white/green ratios were lower at 21 days. The white/red ratio also decreased in the green and blue light cultured discs but not to the extent of the white/green ratio.

Table 12 shows the photosynthesis rates and ratios of discs from cultures using 60 and 1300 $\mu w/cm^2$ of light after 7 and 52 days. At 7 days the lowest white/green ratio occurred in discs from green low and green high light cultures. The highest ratio was in red and white low light cultured discs. The white/red ratios after 7 days of culture were similar, with the highest ratio found in discs from white high light cultures and the lowest in the red high light cultured discs. After 52 days in culture, the white/green ratio showed the same decrease evident in previous studies. The lowest ratios were in discs from white, blue, and green low light cultures. The highest ratios, that were not minus values, were in discs from white and red high light cultured discs. Green high light discs had the highest white/red ratio and red high light discs had the lowest.

Table II. Photosynthesis rates and ratios in red, green and white light of 500 μw/cm^2 following culture for 7 and 21 days under blue, green, red and white light of 60 μw/cm^2.

A. 7 Days	PHOTOSYNTHESIS (ul O_2/g/hr)			PHOTOSYNTHESIS RATIOS	
CULTURE	GREEN LIGHT	RED LIGHT	WHITE LIGHT	WHITE/GREEN	WHITE/RED
White	750±00	5367±649	8292±451	11.06	1.54
Red	1235±214	5144±994	9837±2705	7.97	1.91
Green	1349±267	5930±486	11035±482	8.18	1.86
Blue	1445±112	5413±759	11078±731	7.66	2.05
B. 21 Days					
White	1606±475	3772±388	7460±618	4.65	1.98
Red	3465±1796	2777±871	8392±3406	2.42	3.02
Green	3397±561	7524±1137	11056±3967	3.25	1.47
Blue	2027±490	7608±365	11402±1235	5.62	1.50

Table 12. Photosynthesis rates and ratios in red, green and white light of of 500 $\mu w/cm^2$ following culture for 7 and 52 days under blue, green, red or white light of 60 and 1300 uw/cm^2.

A. 7 Days

	PHOTOSYNTHESIS (ul O_2/g/hr)			PHOTOSYNTHESIS RATIOS	
CULTURE	GREEN LIGHT	RED LIGHT	WHITE LIGHT	WHITE/GREEN	WHITE/RED
White high	550±135	1784±505	4409±313	8.01	2.47
White low	418±100	4201±1468	10043±618	24.02	2.39
Red high	750±175	3567±141	4500±141	6.00	1.26
Red low	438±137	5495±752	8552±681	19.53	1.56
Green high	1284±1269	5335±1106	7399±1050	5.76	1.39
Green low	1225±250	4426±502	6789±623	5.54	1.53
Blue high	___*	7697±3395	10267±2709	___	1.33
Blue low	859±134	4859±1534	6336±1452	7.37	1.30

* = no apparent photosynthesis

Table 12. (Continued)

B. 52 Days	PHOTOSYNTHESIS (ul O_2/g/hr)			PHOTOSYNTHESIS RATIOS	
CULTURE	GREEN LIGHT	RED LIGHT	WHITE LIGHT	WHITE/GREEN	WHITE/RED
White high	237±00	1212±152	2684±460	11.32	2.21
White low	1784±949	2600±248	4687±1027	2.63	1.80
Red high	1133±121	5156±144	6669±1456	5.89	1.29
Red low	___*	1765±00	4460±180	___	2.53
Green high	2267±00	2044±184	8320±453	3.12	4.07
Green low	2787±1549	5285±512	7534±2822	2.70	1.43
Blue high	___*	3560±820	4780±1121	___	1.34
Blue low	2182±858	3818±1456	5878±2913	2.69	1.54

* = no apparent photosynthesis

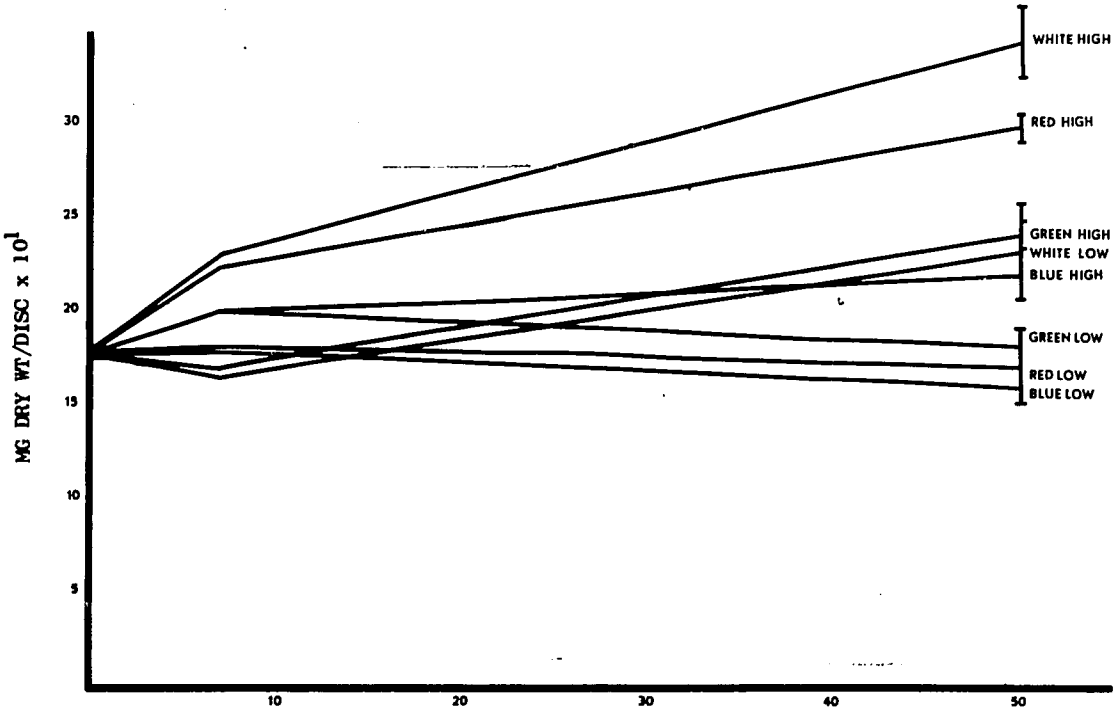
Growth rates. The growth rate of discs from each culture was measured by dry weight measurements after drying for 24 hours at 105° C. The drying was done at various intervals throughout the culture period. The results of the measurements from the respective intensity regime are shown in Figures 11 and 12 and in Tables 8 and 9. Tables 8 and 9 also show the respective photosynthesis rates of the same discs. The results show that the highest growth rates occurred in discs from white and red high light cultures. The discs from green and blue high light cultures, along with discs from white low light cultures, showed slower but similar growth rates. However, the growth rates of these discs were considerably less than the discs from white and red high light cultures. The slowest growth rates were found in discs from red, blue and green low light cultures. The results also indicated that the growth rate was not increased at light intensities higher than 800 $\mu w/cm^2$.

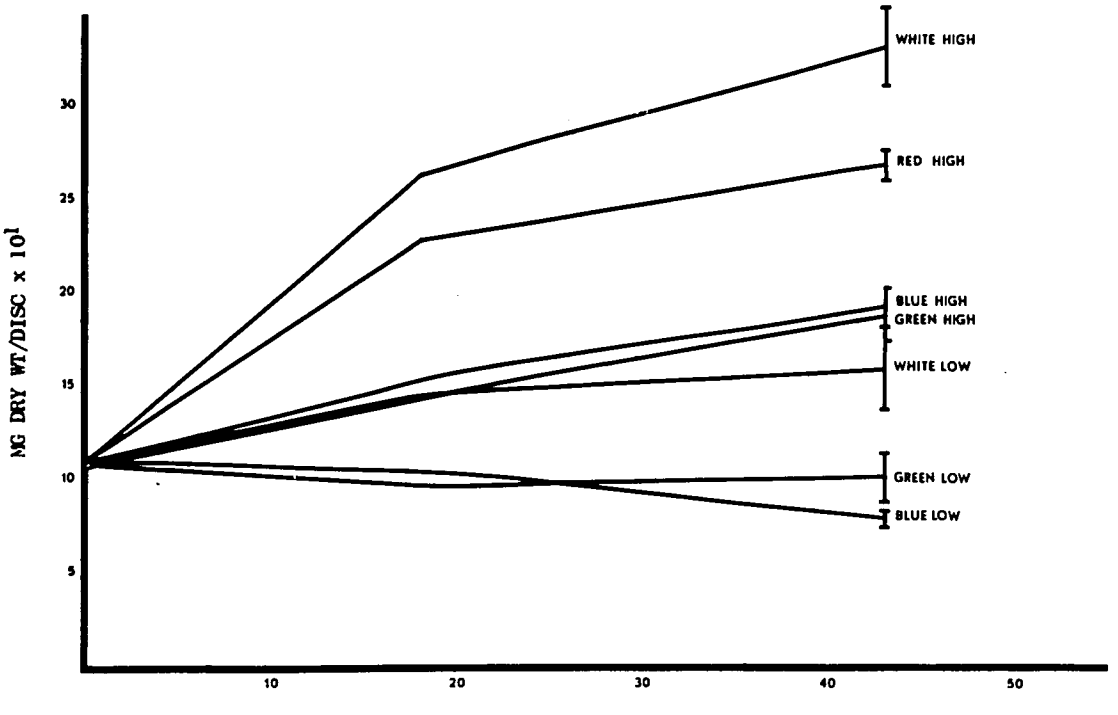
DISCUSSION

The study of the control of pigmentation in algae has usually been discussed in terms of Engelman's theory of "complementary chromatic adaptation", which states that under illumination in a limited spectral region there is a preferential increase in the pigment of highest absorption for the incident wave lengths. There has been much argument about this theory regarding intensity or quality of illumination as the controlling variable (21).

Figure 11. Growth rate of discs in 60 and 1300 $\mu w/cm^2$ light cultures.

Figure 12. Growth rate of discs in 400 and 800 $\mu w/cm^2$ light cultures.


WHITE HIGH
RED HIGH
GREEN HIGH
WHITE LOW
BLUE HIGH
GREEN LOW
RED LOW
BLUE LOW
MG DRY WT/DISC x 10¹
30
25
20
15
10
5
10
20
30
40
50
DAYS IN CULTURE


WHITE HIGH
RED HIGH
BLUE HIGH
GREEN HIGH
WHITE LOW
GREEN LOW
BLUE LOW
MG DRY WT/DISC x 10¹
30
25
20
15
10
5
10
20
30
40
50
DAYS IN CULTURE

The quality of light has been shown to influence the relative proportions and concentrations of pigments in algae. In Anacystis nidulans (17) large changes in the ratio between chlorophyll a and phycocyanin were induced experimentally by control of wavelength of illumination for growth. Hattori and Fujita (13), working with Tolypothrix tenuis, reported that the amounts of phycocyanin and phycoerythrin are controlled by the quality of light, not by the intensity. In other words, growth in green light favored the production of phycoerythrin. Growth in red light favored the production of phycocyanin. They reported that no changes in chlorophyll or carotenoid concentrations occurred. Later work by these same investigators demonstrated a photochemical interconversion between precursors for the two phycobilins. The rate of interconversion was proportional to the light intensity.

The controlling influence of intensity on the concentrations and ratios of pigments has also been demonstrated. Myers and Kratz (17) reported high biliprotein concentrations occurred in algae cultured in low intensity white light. In Chlorella (16) the chlorophyll concentration decreases with increasing light intensity. In Porphyridium (3) the concentration of both chlorophyll and phycoerythrin can be reduced by the increased intensity of white light. The control in these cases was inverse; a decrease in active pigments in response to a general increase in intensity and is often referred to as negative chromatic adaptation.

The acceptance of the two photosystems hypothesized

by Emerson (5) has given new significance to the accessory pigments as collectors and conveyors of light quanta. Consequently, an inverse chromatic control must now be considered. Since both photosystems must be stimulated for normal photosynthesis to occur, the intensity and quality argument has a new complication. Chlorophyll a is the major absorber for PS I. Phycocyanin and phycoerythrin are the major absorbers for PS II. At wave lengths predominately absorbed by phycoerythrin of PS II, quanta are absorbed in excess of that available to drive PS I. At high light intensities of monochromatic light, the photosynthesis rate would be limited by the rate of the photosystem which absorbs least in that spectral region (22). At low intensities of monochromatic light, the rate is limited both by the low intensity and by the rate of one of the photosystems. In low intensity green light a build-up of phycoerythrin would be expected. High intensity green light, according to negative chromatic adaptation, would cause a reduction in the amount of phycoerythrin. The rate of photosynthesis would be limited by the ability of PS I to absorb in this spectral region, and an increase in the relative chlorophyll concentration would be expected. The change would increase the CHL/PHY ratio. This hypothesis is verified by the lowered CHL/PHY ratio in blue light observed in Porphyridium cruentum (3). The hypothesis that the ratio of chlorophyll a to accessory pigments will vary in response to the spectral character of illumination in a manner related to the enhancement spectra for photosynthesis.

The results of this study clearly show the three methods of pigment alteration which were previously mentioned. With respect to light quality and the PE/PC ratio, results similar to those of Hattori and Fujita (13) were found. Red light at low intensities favored phycocyanin formation. At the lower light intensities the PE/PC ratio was lower in discs from the red light cultures than in discs from the low intensity green light cultures. The discs from the blue light cultures also had enlarged PE/PC ratios. This may be accounted for by the spike of green light transmitted by the blue filters used (Figure 1).

The second method of pigment control to be considered is that of light intensity. The results of this study show that when white light is used, the total pigment concentration is sensitive to the intensity of that light. The discs from low intensity white light had higher pigment concentrations than their counterparts from the high intensity cultures. This lower pigment concentration was due to reduced chlorophyll a and reduced phycobilins. When using isolated parts of the visible spectrum, the intensity effect is modified. Even at the highest intensities used in these studies, 2400 $\mu w/cm^2$, the total pigment concentration of discs from blue and green light cultures had almost as much pigment as the blue and green low light cultured discs. In all cases they had more than discs from red and white high light cultures. The discs from the red high light cultures showed pigment reduction similar to that of discs from white high light cultures.

The reason for this pattern of adaptation is evident when considered in relation to the two photosystems and growth rates of these discs. Discs kept in red and white light cultures are sensitive to the light intensity because both photosystems are being irradiated and normal photosynthesis and growth may occur. When the light intensity exceeds the saturation point of photosynthesis, then a decrease in pigment concentration would occur to adjust the rate of the light reaction to that attainable by the dark reaction. The discs from the blue and green cultures cannot be considered in the same manner. In the blue and green high light cultures, the photosynthesis rate is being limited by the complementary photosystem. For these discs, light of the proper wave length is still limiting photosynthesis and growth. In blue and green low light cultures, the photosynthesis rate is being limited by the low level of light quanta for both photosystems. In these cases the results show the third pattern of chromatic adaptation which is similar to the enhancement spectra of photosynthesis. The photosystem limiting maximum photosynthesis should have a relative increase in pigment with regard to the other photosystem. Since the growth rate is slow at high and low intensity green or blue light, discs grown under either of these spectral areas would not be expected to have a total pigment reduction. The slow growth rates also indicate that major adjustments to facilitate higher photosynthesis rates do not occur. The pigment changes should give a higher CHL/PHY ratio in the green high light cultured discs as chlorophyll a absorption is limiting the

photosynthesis rate. The green low light cultured discs could show a similar response but not to the same extent, because the low light intensity by itself is also limiting photosynthesis. Discs from the blue light cultures should show lower CHL/PHY ratios indicating pigment adjustments similar to the enhancement effect of photosynthesis.

The results of this study show that the enhancement of the pigment needed for maximum photosynthesis does occur. The discs cultured in green light had higher ratios of CHL/PHY than the discs cultured in blue light. In addition, discs cultured in high intensity green light had higher CHL/PHY ratios than discs cultured in low intensity green light. Discs cultured in blue high intensity light also had low CHL/PHY ratios. However, this adaptation and its consequences are most clearly demonstrated by the maximum photosynthesis measurements, and by the monochromatic light measurements. They give the best examples of the balancing of photosystems and the adaptation which has occurred as a result of the quality and intensity of light. The highest rates of photosynthesis were always in discs from low intensity cultures, because these discs had the most pigment available and the best balance of the two photosystems. In white and red high light cultures, the light was above the saturation point and the decrease in pigment as well as an unbalance of the photosystems resulted in the lowest photosynthesis rates. In the discs from the blue and green light cultures, only one photosystem is saturated and production of pigment in the other photosystem is enhanced. In these discs the maximum photo-

synthesis rate is higher than in discs from the red and white light cultures. However, it is not as large as in the blue and green low light cultured discs. The differences could be due to pigment concentrations alone. However, in the majority of cases the pigment concentrations are very close, yet the maximum photosynthesis rates are very different. The differences in the photosynthesis rates can best be explained by assuming a more efficient photosynthesis apparatus probably due to a better balance of the photosystems.

The results of the photosynthesis measurements using the monochromatic light gives an additional insight into the alteration and balancing of the photosystems. As previously mentioned, the rates when using blue light were influenced by a large increase in O_2 uptake and were not significant here. The rate of photosynthesis in red light of 500 $\mu w/cm^2$ is approximately 0.6 that measured in white light, of the same energy, for freshly collected plants. The average curve is indicated in Figure 10. The spectral distribution of the red filter allows both PS I and PS II to be stimulated so that near normal photosynthesis can occur. The discs from white and red high light cultures showed little or no photosynthesis when illuminated with green light. This is often the case with freshly collected plants. In discs from the other cultures, the low light cultures and blue and green high light cultures, there is a lowering of the white/green photosynthesis ratio with increasing time in culture. The lowering of the ratio with time indicates an increased ability

to carry out photosynthesis in green light with respect to the photosynthesis rate in white light. The ratio is lowest in discs from blue and green light cultures. The low ratio of white/green photosynthesis in discs from blue light cultures is due to their ability to photosynthesize in green light. The increased ability to photosynthesize in green light is the result of pigment alterations caused by the conditions under which it was cultured.

SUMMARY

The relative concentration of photosynthetic pigments has been shown to be sensitive to the quality of illumination. The total photosynthetic pigment concentration is sensitive to the intensity of light only when that illumination is spectrally diverse enough to drive both photosystems at a rate in excess of the rate of the dark reaction. If the illumination is in narrow spectral regions, then the photosynthesis rate is limited to that rate maintained by the complementary photosystem. In these cases the photosynthetic pigment concentration is not sensitive to the intensity control. An adjustment of the pigment ratios and concentrations induced by low intensity monochromatic light produces a more efficient photosynthesis apparatus presumably due to a better balancing of the photosystems.

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